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APPLICATION NOTE No.512

Automated SRE and HMW DNA Shearing on the epMotion® 5075 for sequencing on PacBio® Long-Read systems

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Introduction

Automating DNA library preparation is essential to improve throughput and reproducibility in long read sequencing. Generating long DNA fragments usually requires additional shearing instruments as Megaruptor® or g-TUBE–based solutions, adding cost and complexity.
This application note demonstrates the automated mechanical in-tip shearing of high molecular weight (HMW) genomic DNA (gDNA) on epMotion 5075t to generate 15–25 kb fragments for use with the PacBio SMRTbell® prep kit 3.0 or HiFi prep kit 96.
Combined with the upstream purification step and the subsequent library preparation steps, this enables the complete workflow to be performed on a single platform, reducing hands-on time and minimizing the need for additional dedicated shearing instruments.

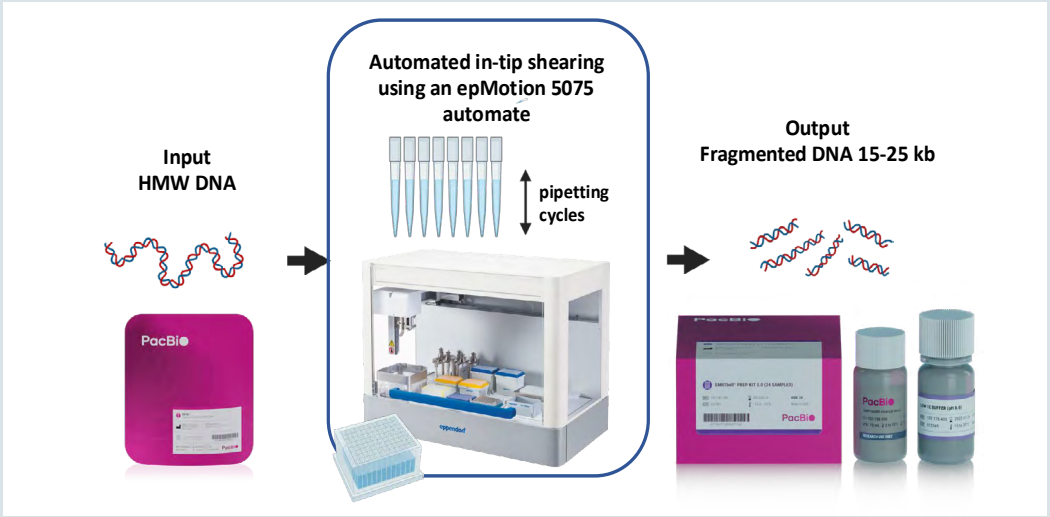

Figure 1: Illustration of automated in-tip shearing on epMotion 5075t liquid handling system using PacBio® SRE kit (PN 102-208-300). Fragmented DNA is suitable for PacBio library preparation using SMRTbell prep kit 3.0 or HiFi prep kit 96.

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PacBio

User benefits

> **Automated in-tip shearing:** Controlled pipetting on epMotion 5075 generates 15–25 kb HMW DNA fragments, replacing dedicated shearing devices and manual fragmentation steps.

> **Consistent Long Read fragment quality:** Optimized pipetting parameters and automation methods ensuring fragment profiles between samples and runs.

> **Ready-to-Use Workflow:** Pre-optimized reagents and validated protocol designed for seamless automation and rapid setup.

> **Flexible all-in-one solution:** A single epMotion 5075t instrument flexibly automates the desired steps – from HMW gDNA purification and shearing through to NGS library preparation – while also supporting many other protocols with step selection based on automation needs.

> **Scalable throughput:** An automation solution that easily keeps pace with increasing PacBio® sequencing demands while maintaining high data quality.

> **Ease of use:** An intuitive, user-friendly system that simplifies set-up and daily operation, reducing training time and risk of user error.

Workflow

The automated workflow on the epMotion 5075t liquid handling system starts with 50 µL of HMW DNA, such as DNA extracted using the fully automated epMotion protocol with the PacBio Nanobind HT CBB kit, and is also compatible with other suitable HMW DNA preparations. The workflow includes an optional short read eliminator (SRE) step to improve DNA quality, followed by shearing of HMW DNA to a size between 15–25 kb and a post-shearing cleanup step. The SRE step comprises an incubation at 50 °C for 1 hour and a 1-hour centrifugation, which are performed off-deck on the Eppendorf Mastercycler® X50s and on the Eppendorf Centrifuge 5920R, respectively.

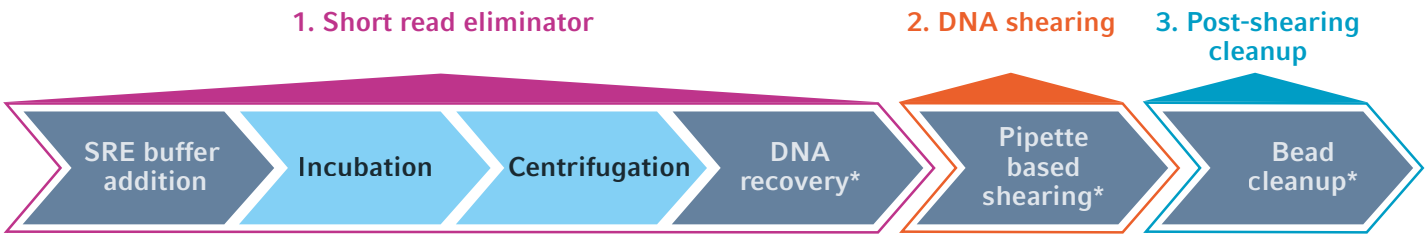

Figure 2: Schematic overview of the DNA shearing workflow with the Eppendorf epMotion 5075t System. Steps shown on dark blue background are performed on the epMotion 5075t liquid handler; steps on a light blue background are carried out off deck. Safe stopping points are present at the end of each step indicated by the asterisks.

Results

Recoveries yield

Using the epMotion® 5075t, 24 high-quality gDNA samples showed post-SRE and post-shear cleanup recoveries within PacBio library prep acceptance ranges, based on concentration measurements using the Qubit™ assay. These results demonstrate that automated pipette-based shearing and cleanup maintain DNA yield within specification.

	DNA overall recovery	
Qualification step	Acceptance criteria	epMotion (n=24)
Post-SRE	65–95%	84%
Post-shearing cleanup	52–90%	71%

Table 1: epMotion® 5075t DNA recoveries. Overall DNA recovery percentage for 24 samples after SRE and post-shear cleanup compared PacBio library prep acceptance criteria.

DNA fragment size

As shown in Table 2, HMW gDNA concentration is one of the parameters that strongly affects the fragment size obtained by automated mechanical in-tip shearing. Thanks to its flexibility and ease of use, the epMotion® 5075t allows multiple shearing methods to be implemented and switched between very easily. To obtain fragment sizes in the 15–22 kb range, with a preferred focus around ~20 kb to improve assembly contiguity and haplotype phasing, it is recommended to use method 1 for input high-quality HMW DNA concentrations of 1–5 ng/µL and method 2 for inputs of 7.5–12.5 ng/µL.

	epMotion method 1 "Shear Low Concentration"	epMotion method 2 "Shear High Concentration"
Input HMW gDNA concentration (ng/µL)	Fragment size (bp) (n=2)	
1	20363	15218
2.5	20551	14955
5	22171	17305
7.5	25015	16744
10	46665	18769
12.5	57143	23216
15	61337	49450

Table 2: Fragment sizes (Agilent Femto Pulse system) obtained from different input concentrations of HMW gDNA using two epMotion® 5075 methods: Method 1 (Shear Low Concentration) and Method 2 (Shear High Concentration).

The epMotion® 5075t protocol includes two in-tip shearing methods with distinct settings adapted to low and high HMW DNA inputs.

Workflow time

Automation enables walk away times of about 85, 110 and 170 minutes for 24, 48 and 96 samples, respectively.

	DNA shearing workflow time (from SRE to cleanup)		
	24 samples	48 samples	96 samples
Automation run time	1h 25m	1h 50m	2h 50m
Hands-on time	0h 20m	0h 20m	0h 20m
Off-deck time	2 h 00m	2h 00m	2h 00m
Total process time	**3h 45m**	**4h 10m**	**5h 10m**

Table 3: SRE and DNA shearing workflow time on the Eppendorf epMotion 5075t for 24, 48, and 96 samples.

Conclusion

The epMotion® 5075 enables fully automated, mechanical in-tip shearing of HMW gDNA and subsequent cleanup with DNA recovery and fragment size distributions that meet PacBio library preparation expectations. By integrating shearing into the same epMotion® platform as the already qualified nucleic acid purification and library preparation workflows, the process reduces instrument needs and hands-on time while supporting scalable, high-quality PacBio® long-read sequencing, as demonstrated in Eppendorf Application Notes 498 and 497.

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